# No evidence for song evolution influencing speciation patterns in honeyeaters

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**Abstract**

Birdsongs are well-known acoustic signals and are thought to play a key role in sexual selection. Despite the established role of birdsong in sexual selection and reproductive isolation at microevolutionary scales, its macroevolutionary impacts on speciation and diversification remain largely unexplored. Here, we test the hypothesis that song influences speciation on macroevolutionary scales, using honeyeaters, a diverse clade of songbirds restricted to Australasia. Using song data for 163 honeyeater species, we employ phylogenetic comparative models of trait evolution to detect shifts in optima for song evolution and use hidden state speciation and extinction models to assess the influence of these shifts on diversification. Contrary to theoretical expectations, we find no evidence that song influences speciation or diversification rates, and models suggest other factors play a role. Overall, song does not significantly contribute to speciation in honeyeaters, likely due to other ecological factors including range size and dispersal ability.

Keywords: sexual signals, birdsong, speciation, macroevolution, RevBayes, trait evolution

## Introduction

Determining what factors cause species diversification is a central goal of many evolutionary studies. The influence of morphological traits (Rabosky et al. 2013; Simões et al. 2020; Cooney and Thomas 2021) and environmental factors, such as ecological opportunity (Mahler et al. 2010; Dumont et al. 2012), on rates of speciation and diversification have been a major focus, but other factors, including sexual signals, can also play a role (Ritchie 2007; Maan and Seehausen 2011; Wagner et al. 2012). Divergence and development of reproductive isolation are key initial phases in speciation events (Coyne and Orr 2004; Hernández-Hernández et al. 2021; Jarvis 2021). Sexual signals frequently emerge as the most divergent traits between closely related species and often experience strong selection (Ritchie 2007; Servedio and Boughman 2017). Evolution and divergence of sexual signals can outpace ecological divergence (Arnegard et al. 2010) and it has been commonly hypothesised that rapid changes in sexual signals could drive species diversification (Maan and Seehausen 2011; Servedio and Boughman 2017).

Vocalisations are in part sexual signals that have the potential to influence speciation. Acoustic signals are used by a wide range of organisms and are complex, behavioural traits that enable individuals of the same species identify one another (Laidre and Johnstone 2013). Acoustic signals have broad-scale functions in species recognition and fine-scale functions in mate choice, thus potentially influencing speciation (Catchpole and Slater 2003; Wilkins et al. 2013). Divergence of acoustic signals has played a role in the early stages of speciation in a wide range of organisms including cicadas (Marshall et al. 2008), crickets (Gray and Cade 2000; Zuk et al. 2006; Tinghitella et al. 2018), frogs (Boul et al. 2007), and birds (Grant and Grant 2006). However, in the context of reproductive character displacement, the strength of selection on signals depends on range overlap, with sexual signals expected to be under greater divergence when species are sympatric rather than allopatric (Maan and Seehausen

2011). Therefore, at macroevolutionary scales (i.e., clade or family-level), the strength of selection on acoustic signals is expected to vary between species and among clades. It remains unclear, however, whether the processes of divergence, assortative mating, and selection of acoustic traits, which occur at the microevolutionary scale, can have macroevolutionary consequences.

Birdsong is one of the best-known acoustic signals and is an important sexual signal. Male birds sing to attract females: consequently, song is hypothesised to act as an important premating isolation barrier in birds and influence speciation (Price 2008; Tobias et al. 2020). The role of birdsong in speciation and reproductive isolation has been studied at fine scales, and empirical evidence supports theoretical expectations. Divergence of song has contributed to reproductive isolation in warblers (Irwin et al. 2001) and has influenced the early stages of peripatric speciation in antbirds in Amazonia (Seddon and Tobias 2007). Additionally, long-term studies on Darwin's finches have demonstrated that sexual selection on differences in vocalisations, resulting from changes in beak shape morphology, have impacted mate choice and contributed to reproductive isolation and speciation (Podos 2001; Podos and Nowicki 2004; Grant and Grant 2006). Although acoustic signals have are commonly hypothesised to influence diversification (Wilkins et al. 2013), most studies focus on population or sister species comparisons, and empirical studies testing the influence of signal divergence on speciation at broader scales remain scarce (Mason et al. 2017). Where the relationship between song and speciation has been investigated at macroevolutionary scales, bursts in rates of speciation coincide with bursts in song evolution, however, no significant correlations have been uncovered at the family-level (Mason et al. 2017), and the influence of song on speciation remains to be explored in other clades and other regions of the world.

The relationship between speciation and birdsong has proven increasingly difficult to disentangle, largely due to song itself being a complex trait, which in birds is influenced by developmental mode and constrained by factors such as beak morphology, body size, and the environment (Derryberry et al. 2018; Friedman et al. 2019; Friis et al. 2022). The majority of macroevolutionary studies on birdsong have therefore focussed on the underlying drivers of song evolution. Like most sexual signals, acoustic signals are expected to diverge between close relatives, especially if ranges overlap, potentially leading to a lack of phylogenetic signal in the trait (Pfennig and Pfennig 2010). However, song frequency is understood to be phylogenetically conserved (Mikula et al. 2021), largely due to constraints imposed by morphological traits, especially body size (Derryberry et al. 2018; Pearse et al. 2018). Moreover, the developmental mode of song is thought to influence song evolution and could complicate its relationship with speciation. Birdsong is either learned or innate, and learning is understood to be a form of phenotypic plasticity which is influenced by cultural transmission and can impact evolutionary outcomes (Pfennig et al. 2010). Learned songs are typically associated with higher rates of evolution (Mason et al. 2017) and divergence (Lachlan and Servedio 2004) than innate songs, although conflicting evidence exists for this (Freeman et al. 2017). Such complexity in the evolution of acoustic traits provides a challenge for speciation approaches and can result in disparity when investigations are conducted over varying phylogenetic and spatial scales.

To overcome such limitations, we test the relationship between song and speciation using a monophyletic clade restricted to a particular region, recognising the power that focusing on a diverse single clade can bring (Marquet et al. 2004). Thus, we focus on honeyeaters (Aves: Meliphagidae), a phenotypically diverse clade of birds (192 species; Gill et al. 2020) that range throughout Australasia and are known for their distinctive vocalisations (Higgins et al. 2008). Song in honeyeaters is learned and varies greatly among

species, with some species exhibiting complex and variable songs such as the Tūī (Hill et al. 2015), while others have simpler and more stereotyped songs, such as the noisy miner (Jurisevic and Sanderson 2016). This variation in vocalisations, combined with the high diversity and endemism of honeyeaters, makes this group an ideal model for studying the evolution of vocalisations and their potential role in speciation. Specifically, we use honeyeaters as a model clade to test the hypothesis that song influences speciation at macroevolutionary scales and that rates of song evolution are correlated rates of speciation. Using a dataset of five song variables for 163 species of honeyeater, we implement an approach where we first model shifts in trait evolution and identify species associated with shifts in song evolution, and test whether these are associated with increased rates of speciation and diversification.

## Methods

*Song and phylogenetic data*

We use a song dataset from a previous honeyeater study (Hay et al. 2023). Containing song data for 163 honeyeater species, the song dataset considers five relevant aspects of song (Fig. 1; Table S1) which have commonly been explored in past song studies (Mason and Burns 2015; Mason et al. 2017; Derryberry et al. 2018): peak frequency (frequency which the most sound energy was produced), maximum frequency (maximum frequency of the highest pitched note in song), minimum frequency (minimum frequency of the lowest pitched note in song), song bandwidth (difference between maximum and minimum frequency), and song pace (number of notes/song duration). Song variables were $\log_{10}$-transformed prior to statistical analysis to meet parametric assumptions of normality and homogeneity of variance, and because logarithmic scales of sound frequency correspond to how birds perceive and modulate sound (Cardoso 2013).

To model trait evolution and estimate speciation rate we used a honeyeater phylogeny from Hay et al. (2022), which contains 192 species of honeyeater. The phylogeny was trimmed to represent the 163 honeyeater species for which we had song data and using the '*drop.tip'* function from the 'ape' package (Paradis et al. 2004) in R (R Core Team 2013). Trait values are predicted to be more similar between closely related species, however sexual signals are often expected to be the most divergent traits between species (Servedio and Boughman 2017). To test for how conserved song traits are, we used the '*phylosig'* function from the 'phytools' package (Revell 2012) in R (R Core Team 2013) and calculated the phylogenetic signal of each song variable, using lambda and a likelihood ratio test. When lambda is equal to 1 variation along the phylogenetic tree is equal to that of a Brownian motion model, whereas values lower than one suggest less variation than expected under Brownian motion (Revell et al. 2008).

*Analyses*

A range of phylogenetic comparative methods, implemented through the program RevBayes (Höhna et al. 2016) were used to test the relationship between song and speciation. RevBayes enables Bayesian inference for phylogenetic comparative methods, and uses a probabilistic graphical model framework which is a powerful generic framework for specifying and analysing statistical models (Höhna et al. 2014; Höhna et al. 2016). All RevBayes models were implemented through the CIPRES Science Gateway v3.3 online server (Miller et al. 2010). First, to explore patterns of song evolution in honeyeaters, we used relaxed Ornstein-Uhlenbeck (OU) models of trait evolution to identify shifts in trait evolution of the different song traits in RevBayes. OU models were also implemented in a multivariate framework using RevBayes and verified using multivariate OU models in the package 'PhylogeneticEM' (Bastide et al. 2018) in R (R Core Team 2013). The influence of these shifts on speciation

173 was then tested with hidden state-dependent speciation and extinction models (Beaulieu and
174 O'Meara 2016) in RevBayes (Höhna et al. 2016).

175 Relaxed Ornstein-Uhlenbeck models of trait evolution were used to model song
176 evolution along the honeyeater phylogeny. Relaxed OU models allow the optimal phenotype
177 to vary over branches of the phylogenetic tree to estimate tip-specific measures of OU
178 evolution (Uyeda and Harmon 2014) and enabled us to infer branch-specific evolutionary
179 optima of song evolution in honeyeaters. Here, we first assessed patterns of OU trait
180 evolution in each of the five song traits (peak frequency, maximum frequency, minimum
181 frequency, bandwidth, and pace) separately. Relaxed OU models were implemented in
182 RevBayes using reversible-jump Markov chain Monte Carlo. Examples from the online
183 tutorial (May and Höhna 2022) were followed, and the MCMC sampler was set for 4 runs of
184 50,000 generations with a burn-in of 10%. Tracer v1.7.1 (Rambaut et al. 2018) was used to
185 visualise log files and to ensure convergence by verifying ESS values were above 200 for all
186 parameters. Because priors can influence performance of OU models, particularly the number
187 of expected shifts, all models were repeated over a number of shifts (15, 30, and 45). Model
188 fit was assessed using stepping-stone and path sampler analyses, and the best model was
189 selected based on marginal likelihood values. In all cases the preferred models had 45 shifts
190 (Table S2). Branch-specific rates of OU evolution were visualised by plotting output using
191 the package 'RevGadgets' (Tribble et al. 2021) in R (R Core Team 2013). Branch theta
192 values were extracted for each species and shifts in OU trait evolution were determined based
193 on plots and theta values (Table S3).

194 Trait evolution is inherently a multivariate process (Walsh and Blows 2009; Revell
195 and Harmon 2022). Thus, we also used multivariate relaxed OU models in RevBayes. The
196 function '*dnPhyloOrnsteinUhlenbeckREML'* for the relaxed OU model in RevBayes does not

enable the inclusion of a correlation matrix to account for covariation between multivariate traits. To overcome this limitation, we performed a phylogenetic principal component analysis on the five song variables to ensure no correlation between traits, and considered PC1 to PC3, which accounted for 97.4 % of the total variation, in analysis. Multivariate model parameters, model fit, and visualisation followed the same approach and settings as univariate models above.

As an additional measure to test for shifts in trait evolution, and to verify the results from the multivariate relaxed OU models in RevBayes, we also used the package 'PhylogeneticEM' (Bastide et al. 2018) in R (R Core Team 2013). PhylogeneticEM simulates a scalar OU model of evolution that allows for maximum likelihood estimation of shifts in OU trait evolution and uses this approach to automatically detect position of shifts along a phylogeny, while accounting for correlations among traits (Bastide et al. 2018). The function '*phyloEM'* was used to test for OU shifts in multivariate trait evolution, using PC1-PC3, the same dataset which was used for the multivariate OU model in RevBayes.

Finally, to test the influence of shifts in song evolution on speciation rates in honeyeaters we used hidden state-dependent speciation and extinction models (HiSSE; Beaulieu and O'Meara 2016) which were also implemented in RevBayes (Höhna et al. 2016). The HiSSE framework enables testing of character dependent diversification and additionally models an unobserved hidden character that potentially impacts species diversification rates. HiSSE relies on testing the influence of binary characters on speciation but can disentangle rate variation due to the character of interest with a hidden state. HiSSE represents a substantial improvement on previous state-dependent diversification models (Maddison and FitzJohn 2015; Rabosky and Goldberg 2015). Species associated with shifts in song evolution were coded with a '1' and all other species were coded as '0'. This was done to test whether

221 shifts in song evolution are also associated with increases in diversification rate. Speciation
222 models can be subject to bias when species are missing (Mynard et al. 2023), therefore we
223 implemented all HiSSE models across the full honeyeater phylogeny containing all 192
224 species of honeyeater (Hay et al. 2022). The 29 honeyeater species with no song data were
225 treated as having unknown tip states and were coded with '?' rather than '0' or '1'. The
226 MCMC sampler was set for 4 runs of 3,000 generations with a burn-in of 10%. Tracer v1.7.1
227 (Rambaut et al. 2018) was used to visualise output and determine convergence by verifying
228 ESS values were above 200 for all parameters.
229
230 **Results**
231 Phylogenetic signal varied substantially among the five song variables. Values of lambda
232 varied from 0.27 to 0.91 and the significance values of the likelihood ratio tests were all
233 below $p < 0.001$ (Table S4). Song bandwidth had the lowest signal ($\lambda = 0.27$), followed by
234 maximum frequency ($\lambda = 0.61$), pace ($\lambda = 0.68$), peak frequency ($\lambda = 0.75$) and then
235 minimum frequency ($\lambda = 0.91$).

236 Investigation of the evolution of song variables with univariate relaxed OU models
237 revealed several patterns. Overall, the three frequency variables (peak frequency, maximum
238 frequency, and minimum frequency) had very similar estimates for relaxed models of OU
239 trait evolution. Here, the clade *Myzomela* + *Sugomel* is associated with a shift in OU trait
240 evolution for all three frequency variables, and *Philemon* is identified with shifts in peak and
241 maximum frequency (Fig. 2). Despite this, we uncovered little evidence that shifts in trait
242 evolution influence speciation rates for peak frequency (mean $\lambda_{no\ shift} = 2.44$, 95 % CI = [1.80,
243 3.18]; mean $\lambda_{shift} = 3.46$, 95 % CI = [2.31, 4.74]; Fig. 2; Table S5), maximum frequency
244 (mean $\lambda_{no\ shift} = 2.49$, 95 % CI = [1.83, 3.23]; mean $\lambda_{shift} = 3.17$, 95 % CI = [2.18, 4.28]; Fig.

2; Table S5), and minimum frequency (mean $\lambda_{no\ shift}$ = 2.55, 95 % CI = [1.88, 3.24]; mean $\lambda_{shift}$ = 3.35, 95 % CI = [2.10, 4.77]; Fig. 2; to S5). Shifts in bandwidth (mean $\lambda_{no\ shift}$ = 2.78, 95 % CI = [2.11, 3.48]; mean $\lambda_{shift}$ = 1.56, 95 % CI = [0.50, 2.93]) and pace (mean $\lambda_{no\ shift}$ = 2.81, 95 % CI = [2.08, 3.57]; mean $\lambda_{shift}$ = 1.45, 95 % CI = [1.01E-6, 5.33]) appear to be associated with lower rates of speciation (Fig. 2; Table S5), but this is also non-significant, and could be due to the lower sample sizes for shifts in bandwidth (14 species associated with shifts) and pace (7 species identified with shifts).

The two methods of multivariate OU trait evolution (RevBayes and PhylogeneticEM) uncovered similar shifts in optima of song in honeyeaters. Species from the genera *Myzomela*, *Sugomel*, *Philemon*, *Nesoptilotis* and *Gymnomyza* are all associated with shifts in OU trait evolution in both approaches (Fig. 3). The PhylogeneticEM method does reveal a shift associated with the larger clade containing *Melidectes* and relatives, which was not recovered in the RevBayes relaxed multivariate OU model (Fig. 3).

We found no evidence that shifts in song evolution are associated with increased rates of speciation or net diversification for both the RevBayes (mean $\lambda_{no\ shift}$ = 2.54, 95 % CI = [1.81, 3.31]; mean $\lambda_{shift}$ = 3.03, 95 % CI = [2.09, 4.00]; mean $diversification_{no\ shift}$ = 2.52, 95 % CI = [1.78, 3.29]; mean $diversification_{shift}$ = 2.98, 95 % CI = [2.05, 3.98]) and PhylogeneticEM approaches (mean $\lambda_{no\ shift}$ = 2.24, 95 % CI = [1.53, 3.01]; mean $\lambda_{shift}$ = 3.29, 95 % CI = [2.28, 4.44] ; mean $diversification_{no\ shift}$ = 2.21, 95 % CI = [1.50, 2.99]; mean $diversification_{shift}$ = 3.25, 95 % CI = [2.28, 4.47]), and the 95 % confidence intervals overlap substantially (Fig. 3; Table S5). Both methods uncovered similar estimates for speciation and net diversification (Fig. 3; Table S5).

## Discussion

In this study, we used honeyeaters to explore the macroevolutionary consequences of song evolution, a sexually selected behavioural trait, which has commonly been hypothesised to influence speciation in birds. We find no evidence that shifts in song evolution are associated with increases in speciation or diversification rates. The significant overlap between the confidence intervals of states is present in all tests, and hidden state speciation and extinction models suggest that there are other factors (hidden states) which play a role. Ultimately, song does not appear to have contributed to speciation on a macroevolutionary scale in honeyeaters, possibly due to other factors including geographical processes and dispersal to islands dominating speciation history in honeyeaters (Hay et al. 2022), and any apparent influence of song is likely mediated by these factors and underlying variation in body size.

Most of the interspecific diversity of song frequency and pace in honeyeaters can be explained by evolutionary history. Theory suggests that divergence is expected for sexually selected traits between closely related species (Maan and Seehausen 2011; Servedio and Boughman 2017). Thus, the level of phylogenetic signal uncovered for frequency and pace is contrary to such theoretical expectations. This is unsurprising, however, given other studies have found similar levels of phylogenetic signal for song traits in birds. For example, peak frequency was found to have a lambda of 0.87 for 5085 species of passerine birds (Mikula et al. 2021), and a lambda of 0.74 for 1022 passerine species (Friis et al. 2022). Our results are consistent with these previous studies and confirm that peak frequency of song is phylogenetically conserved. This is likely driven by allometric constraints imposed by body size (Fletcher 2004), which itself is a very conserved trait, and directly impacts the length of the vocal tract and the size of the syrinx (Bertelli and Tubaro 2002; Suthers and Zollinger 2008). Certainly, body size has been demonstrated to have a prominent influence on honeyeater song and has been found to structure variation in frequency and song pace (Hay et al. 2023). Interestingly, minimum frequency was found to have the highest phylogenetic

signal rather than peak frequency. It is unclear if this is the case in other clades as past studies have typically focussed on peak frequency. Nonetheless, body size is understood to place a limit on the lowest frequency that a bird can effectively produce (Fletcher 2004; Friis et al. 2021), so perhaps minimum frequency is just particularly constrained by body size.

We found no evidence that shifts in song evolution are associated with higher rates of speciation and net diversification. Macroevolutionary influences of song evolution on speciation have been explored in 581 species of tanagers and ovenbirds and shifts in song were found to be correlated with shifts in diversification, but this was not significant when each family was considered separately (Mason et al. 2017). This is consistent with the findings of this study, which is restricted to the family-level, and could imply that macroevolutionary signals of song on speciation and diversification emerge only when very different clades of birds are examined simultaneously, as is the case for other taxa too (Rabosky et al. 2013; Rabosky and Huang 2016). Additionally, the song dataset used in this study was missing data for 29 honeyeater species. As is often the case with missing data, species inhabiting the most remote areas were data deficient. This skewed our dataset towards common species and especially those found in Australia and New Zealand. Areas such as New Guinea, which has high honeyeater species richness (Marki et al. 2019), and islands throughout the Indo-Pacific, which contain endemic species, were lacking in data. Given previous work highlighting island settings for speciation in the group (Hay et al. 2022), this potential effect of our data set should be considered a caveat for the study.

Nonetheless, our results indicate that song is not a major driver of speciation at macroevolutionary scales in honeyeaters. Recent studies have found that faster rates of song evolution do not result in higher speciation rates in New World passerine birds (Freeman et al. 2022), and ecological and demographic factors may provide a better explanation for

speciation (Singhal et al. 2018; Harvey et al. 2019; Freeman et al. 2022). State-dependent speciation and extinction models used in the present study indicate hidden states are associated with differential rates of speciation and net diversification, implying that other factors not considered here could contribute to patterns of speciation. Honeyeaters have a dynamic evolutionary history: Australia is the centre of honeyeater diversity (Driskell and Christidis 2004; Norman et al. 2007; Oliveros et al. 2019), however, dispersal to islands throughout the Indo-Pacific, and vicariant speciation due to connection and disconnection of New Guinea and the changing biomes of Australia have played key roles in the diversification of this group (Toon et al. 2010; Andersen et al. 2014; Marki et al. 2017; Hay et al. 2022). Moreover, past studies have found range size and dispersal ability to significantly contribute to speciation in honeyeaters when applying state-dependent speciation and extinction models (Hay et al. 2022). Allopatric speciation is the predominant mode of speciation in honeyeaters and could explain the lack of influence of song evolution on speciation because selection on signal divergence is expected to be stronger for sympatry (Pfennig and Pfennig 2010). Songs are thought to diverge slowly in allopatry, and only rapidly increase on secondary contact, contributing to reinforcement (Lachlan and Servedio 2004; Servedio and Boughman 2017). Thus, contributions of song to speciation likely depends on the mode of speciation, so although song could be aiding microevolutionary processes such as reproductive isolation, other factors including range size and dispersal ability have contributed more to speciation more at the macroevolutionary scale (Hay et al. 2022).

Across all models of trait evolution, shifts in song evolution are notable in clades of *Myzomela* + *Sugomel* and *Philemon*. These clades have previously been identified to have increased rates of speciation (Hay et al. 2022) and are also the most speciose genera in honeyeaters (Gill et al. 2020). However, past studies have shown that small range sizes

resulting from dispersal to islands has been a primary driver of speciation in these clades (Hay et al. 2022), and the (non-significant) slight increase in speciation rate observed with these shifts in song evolution could be due to these underlying factors. It is also important to consider the influence of body size on these patterns because honeyeater song is under strong allometric constraints (Hay et al. 2023), and these clades show great differences in body size. For example, all species of *Myzomela* are between 6 to 16 g, whereas species of *Philemon* range from 50 g to 194 g, in which the majority of species are over 100 g (Dunning 2008; Tobias et al. 2022). Additionally, body size evolution has been correlated with speciation and species diversity across different vertebrate clades (Ricklefs 2004; Rabosky and Adams 2012; Rabosky et al. 2013) and has contributed to speciation in honeyeaters (Brennan and Keogh 2018; Marki et al. 2019). Therefore, it is possible that the shifts in song evolution could be due to shifts in body size evolution, and underlying variation in body size could be contributing to both the observed patterns of song evolution and speciation in this study.

Understanding the influence of reproductive isolation on diversification has been recognised as an urgent question in evolutionary biology (Matute and Cooper 2021), especially because the evolution of reproductive isolation often defines speciation (Coyne and Orr 2004). Here, we contribute to understanding of this question by testing the hypothesis that song has influenced speciation on macroevolutionary scales in honeyeaters. We uncovered several shifts in song evolution but found no evidence that these shifts were associated with significant increases in diversification or speciation rates. Models also indicated that other factors (hidden states) contribute to differential rates of speciation and diversification, implying that speciation history in honeyeaters is better explained by other factors.

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## Figures

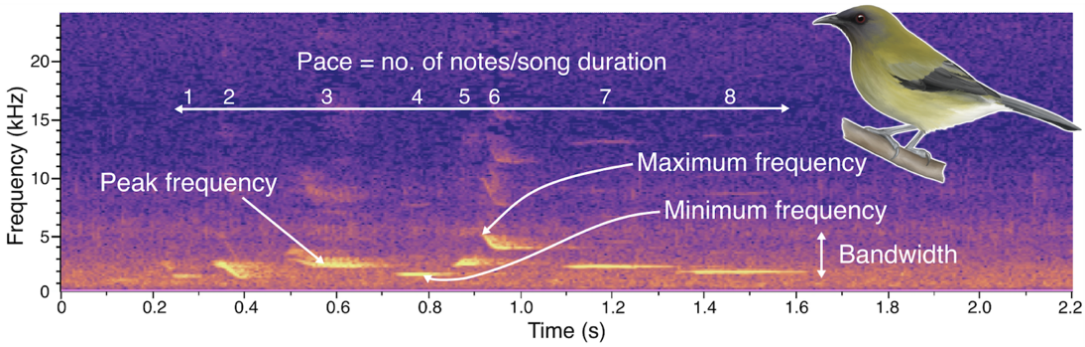


**Figure 1.** Song spectrogram for the New Zealand Bellbird (*Anthornis melanura*; xeno-cento recording accession number: XC153005), displaying frequency (kHz) over time (s). The five song variables considered in this study are indicated with annotations; peak frequency represents the frequency of maximum amplitude across the entire song, maximum frequency is the highest pitched note in song, minimum frequency is the lowest pitched note in song, bandwidth is the difference between maximum and minimum frequency across the song, and pace is the number of notes over song duration. NZ Bellbird illustration by Eleanor Hay.

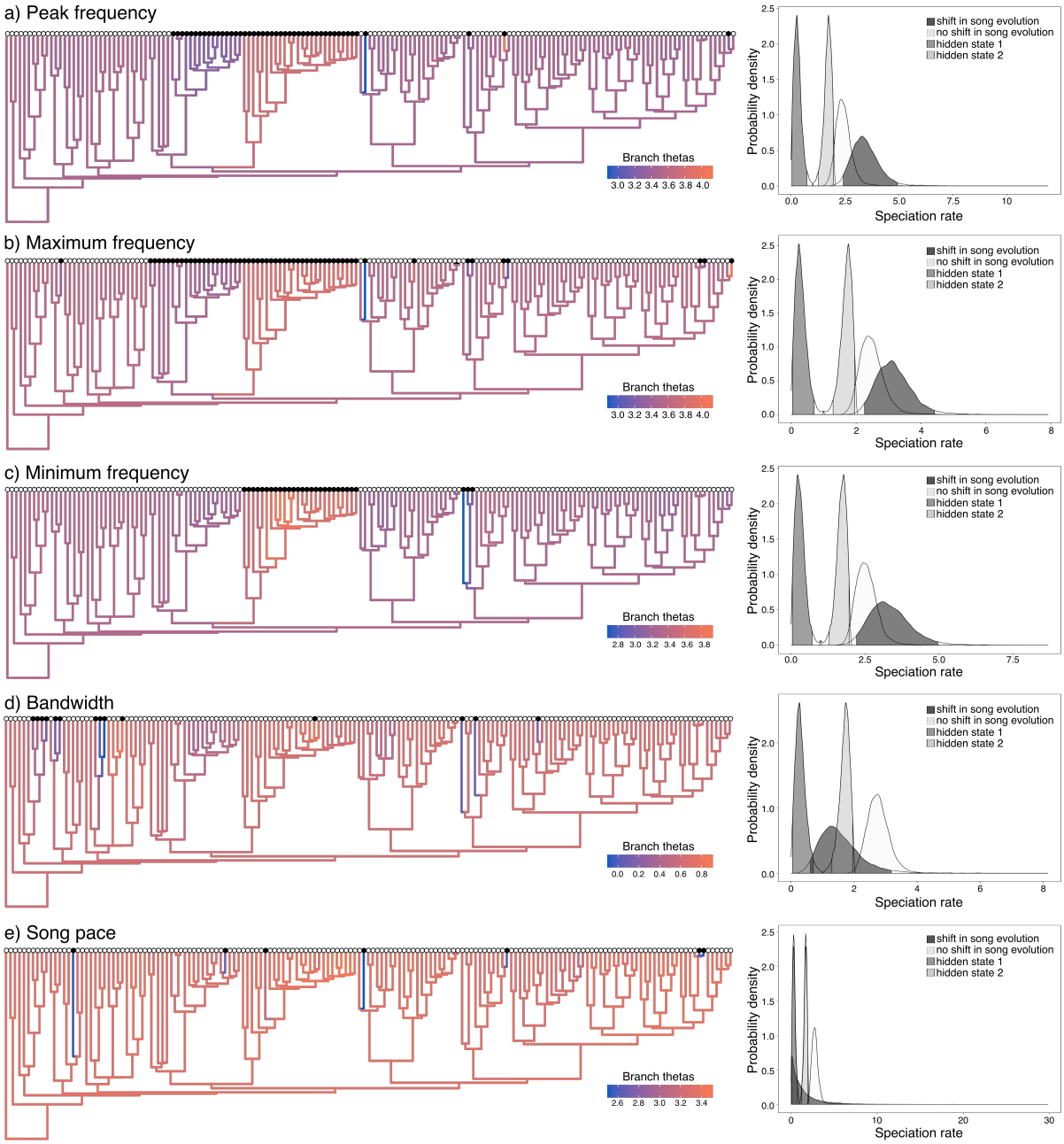


**Figure 2.** Results from relaxed OU and HiSSE models for each of the five song traits; peak frequency (a), maximum frequency (b), minimum frequency (c), bandwidth (d) and pace (e), Phylogenetic trees display branch specific theta values from relaxed OU models in RevBayes. Dots at the tips of the trees indicate which species were identified as associated with shifts in song evolution and were tested to influence speciation rates. Respective speciation rates extracted from HiSSE models associated with the coding at the tips are displayed in the density plots to the right of each phylogeny.

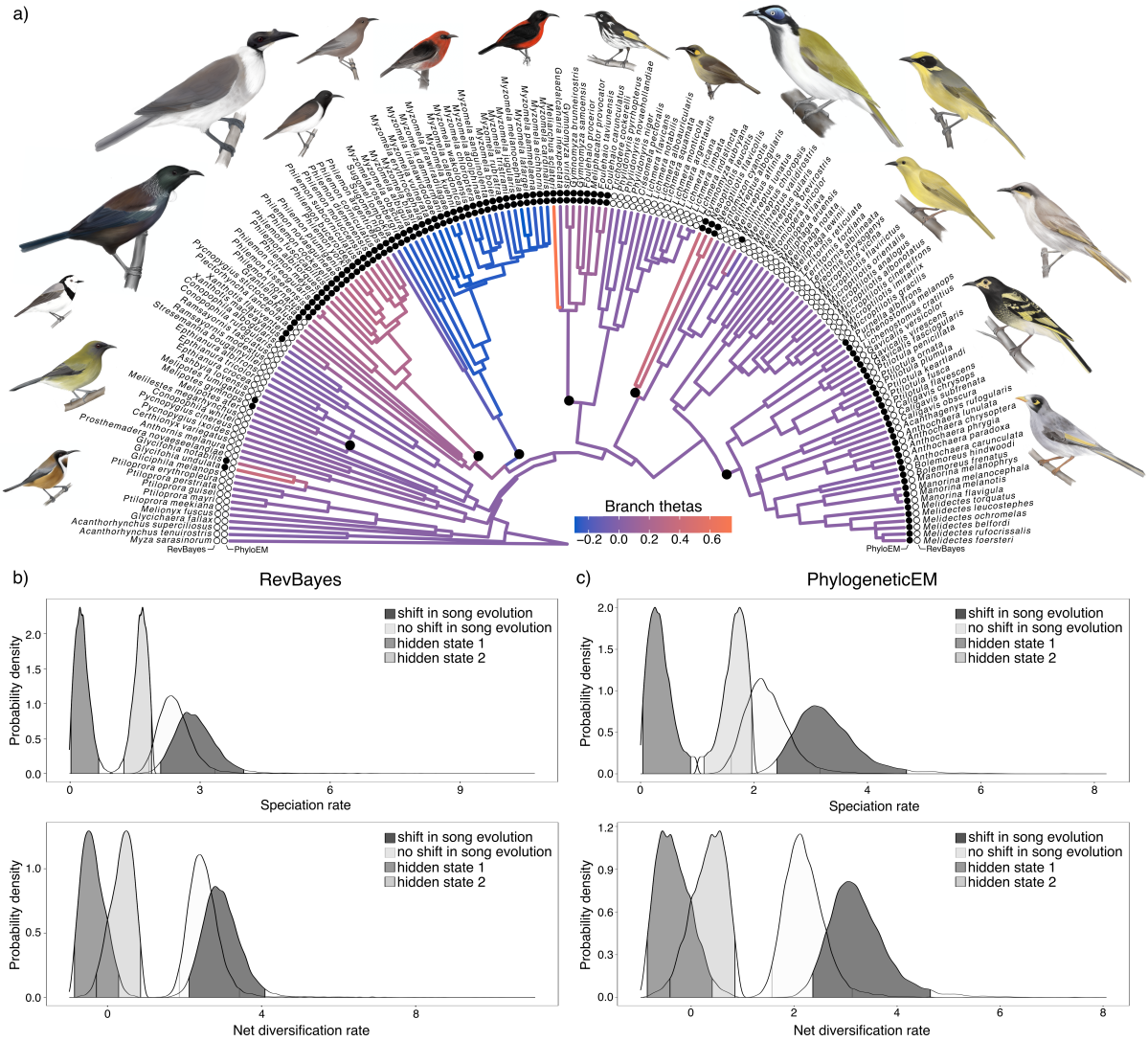


**Figure 3.** Honeyeater phylogenetic tree (a), the branches of the phylogeny display estimated branch theta values from the multivariate relaxed OU model in RevBayes. Dots on the branches of the phylogeny indicate shifts revealed by PhylogeneticEM. Dots at the tip of the tree show the binary classifications for HiSSE models from the RevBayes and PhylogeneticEM (labelled as PhyloEM) approaches. Density plots below the phylogeny show the estimated speciation and net diversification rates from HiSSE models for shifts identified by RevBayes (b) and PhylogeneticEM (c). Birds are illustrations by Eleanor Hay.